996,089

PATENT SPECIFICATION

DRAWINGS ATTACHED

996,089

Date of Application and filing Complete Specification Feb. 15, 1963.
No. 6321/63.

Application made in Germany (No. B65959 IVa/30h) on Feb. 15, 1962.

Complete Specification Published June 23, 1965.

Index at acceptance: —A2 D(2S, 3A)

Int. Cl.:—A 01 n 1/00

COMPLETE SPECIFICATION

Improvements in or relating to Methods of Preserving Blood

We, "BIOTEST"-SERUM-INSTITUT G.M.B.H a German Body Corporate of Flughafenstrasse 4, Frankfurt/Main-Niederrad—Germany, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method of preserving blood, in particular, erythrocytes (red blood cells).

According to the invention there is provided a method of preserving blood, particularly liquids comprising erythrocytes wherein at least a portion of the intracellular metabolic products of the red blood corpuscles, which accumulate in the red blood cell under the storage conditions, are removed from the erythrocytes during storage and/or intercepted within the red blood cell.

In the accompanying drawings Figure 1 is a lactic acid determination graph wherein E=extinction and T=absolute temperature;

Figure 2 is a graph for comparing speed of movement of toxic metabolic products with temperature wherein T=absolute temperature and K=reaction constant;

and Figure 3 is a graph of lactic acid concentration in suspension medium wherein C=temperature in degrees centigrade and %=percentage concentration of the products of metabolism within the cell in contrast to the extracellular phase.

Blood is generally stored, particularly in the form of preserved blood, using the stabilising ACD-solution (USP XVI). The ACD-solution contains citrate as an anti-coagulant and glucose for maintaining the glycolysis of the blood to be stored. The ACD-solution also contains, for reasons connected with autoclaving, citric acid, in order to prevent the sugar from caramelising.

The blood thus preserved can generally be stored up to 21 days, after which a rapid haemolysis of the red blood cells sets in and the blood loses all its physiological value. Repeated researches have been made with the object of increasing storability; in particular it has hitherto been necessary to reject erythrocyte sediment i.e. separated erythrocytes, such as occur in large quantities in the course of the production of plasma preserves, because of the inadequate storability of erythrocyte, in spite of the important ethical and economical arguments against such action.

To improve storability of the red blood cells different ways have been tried. One is the addition of nucleosides, such as adenosine, inosine or guanosine to the ACD-solution. But a separation then takes place into the purine constituent (for example adenine, hypoxanthine, guanine) and the ribose constituent, the latter being phosphorylated in the 1-position. This ribose-1-phosphate is then metabolized by the red blood cells and converted to lactic acid or decomposed by oxidation to CO_2. No essential improvement in storability has been obtained.

Another method described has been storage at low temperatures (−20°C to −200°C), in glycerol which has made it possible to obtain a storage period of 8 months. The drawback of the method is the removal of glycerol necessitated after the thawing-out and the high haemolyse ratio of about 15% connected with this procedure.

Actually, in the blood circulation under normal physiological conditions the erythrocytes have a life expectancy of about 120 days, that is 0.83% of the total population dies every day in the body at a temperature of 37°C. If blood is stored at a lower temperature (+4°C) then the cell metabolism runs down at a speed which is only 1/1000th of the speed at 37°C, as is clear from the determination of lactic acid after incubation at various temperatures shown in Figure 1.

Here the logarithm of the extinctions measured for the lactic acid formed is plotted as a function of the inverse value of the absolute temperature. The theoretical storage time of erythrocytes at a temperature of 4°C will therefore be 1200 days. Contrasted with this theoretical storage time of 1200 days the results obtained hitherto (less than 30 days) are very poor.

It has been found in the course of researches that as the temperature falls, the speed of movement of the toxic metabolic products through the cell walls drops considerably faster than the velocity of the metabolic reactions. In fact at low temperatures this difference is quite considerable, as can be seen from the results of measurements shown in Figure 2, for metabolic velocity and speed of movement, in dependence on the temperature. Curve *a* shows the course of lactic acid formation and curve *b* the penetration speed of anions in dependence on the inverse value of the absolute temperature.

In the light of the invention, it has been shown that the concentration of the metabolic products in the red blood cells, which can be measured directly, is very important. The concentration of the lactic acid in the red blood cells accumulates 2 to 3 fold over the content of lactic acid in the suspension medium. (Figure 3).

Now, it is known from cell physiology that a pH displacement towards the acid side results in a decline in the essential hexokinase reaction of the cell metabolism. The enrichment of lactic acid, for instance, inside the cell is likewise a cause of a considerable pH displacement and hence responsible for the early decay of the red blood cell.

Care must be taken, to ensure that the intracellular concentration of metabolic products normally occuring during storage is prevented. Long-term storage of blood or any other erythrocyte preparation is therefore successfully achieved in accordance with the invention if at least a portion of the intracellular metabolic products of the erythrocytes (e.g. lactic acid) is removed from the erythrocytes during storage and/or intercepted inside the erythrocytes. During any deleterious concentration of the metabolic products must be prevented by a continuous or intermittent action.

This can be effected in any manner known to the person skilled in the art, for instance, the metabolic products are removed by diffusion through the cell wall of the erythrocyte.

The metabolic products in the erythrocytes can also be intercepted, i.e. trapped and detoxified by combination or modification. Another way to diminish the accumulation of metabolic products comprises the retardation of the metabolism of the erythrocytes in such a manner that the concentration of metabolic products only reaches dangerous proportions after longer periods than with conventional methods.

The desired result can be achieved by feeding appropriate adsorbents in suitable quantity to the blood to be stored. The adsorbent will maintain a permanent high diffusion gradient between the interior of erythrocytes and the suspension medium. The blood can also be stored for instance in containers made of a material which is permeable to low-molecular weight substances, that is, the products of metabolism, such as semipermeable membrane. The necessary diffusion gradient to eliminate the metabolic products can be maintained by continuous dialysis against a suitable liquid such as buffered saline (an analogous case is that of artificial kidneys by which the metabolic products of flowing blood can be secreted *in vivo*).

For the dialysis liquid there may be advantageously used a suitable buffer system which maintains the hydrogen ion concentration of the blood contant during the storage period (for example a Sorensen buffer substance with a pH=7.4) and which also contains sufficient sugar (for example, glucose) and phosphate.

Furthermore there may be added any desired additives which exert a favourable influence on the life term of the erythrocytes, e.g. vitamins, coferments and nucleosides. In particular these additives can be added in the course of storage of the blood at any desired time. For example, it would be best to add nucleosides only in the second half of the storage period, as their efficacy begins only from this point of time.

The whole dialysis process during storage can be automated by providing suitable devices, for instance in a refrigerator, to carry out the continuous exchange of the dialysing fluid, the addition at given times of substances to the dialysing liquid, and its regeneration.

WHAT WE CLAIM IS:—

1. A method of preserving blood, particularly liquids comprising erythrocytes wherein at least a portion of the intracellular metabolic products of the red blood corpulscles, which accumulate in the red blood cell under the storage conditions are removed from the erythrocytes during storage and/or intercepted within the red blood cell.

2. A method as claimed in claim 1, wherein the metabolic products are removed from the erythrocytes by a high diffusion gradient between the interior of the erythrocytes and the surrounding fluid.

3. A method as claimed in claim 1 or claim 2, wherein the metabolic products are intercepted in the erythrocytes, by chemical modifications or combination, or by retardation of the intracellular metabolism.

4. A method of preserving blood, particularly erythrocyte concentrations, substantially as hereinbefore described.

"BIOTEST"-SERUM-INSTITUT G.M.B.H.
Per: Boult, Wade & Tennant
111/112 Hatton Garden, London, E.C.1.
Chartered Patent Agents.

Leamington Spa: Printed for Her Majesty's Stationery Office by the Courier Press.—1965.
Published at The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.

996089 COMPLETE SPECIFICATION
1 SHEET This drawing is a reproduction of the Original on a reduced scale

Fig. 1.

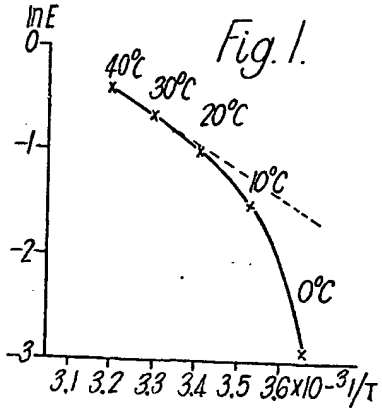

Fig. 2.

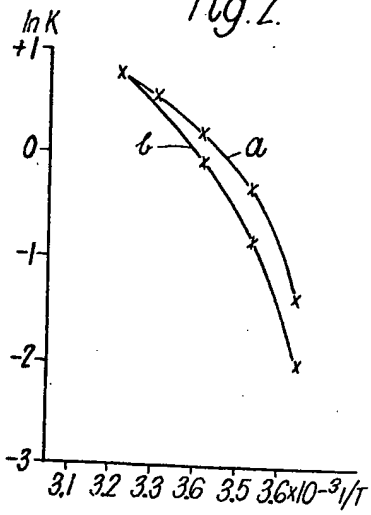

Fig. 3.